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CLINAL VARIATION IN *HESPERIA LEONARDUS* (HESPERIIDAE) IN THE LOESS HILLS OF THE MISSOURI RIVER VALLEY

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ABSTRACT. Specimens of *Hesperia leonardus* Harris were collected from a potential subspecies intergrade area known as the Loess Hills, which are bluffs of xeric prairie extending from the southeastern tip of South Dakota to the northwestern corner of Missouri. Specimens were rated for five characteristics: (1) forewing length, (2) ventral hindwing color, (3) ventral hindwing median band size, (4) dorsal fulvousness of males, and (5) transparency of the dorsal forewing hyaline spot in females. Reference specimens included typical *H. l. pawnee* Dodge and typical Ozark *H. l. leonardus*. Comparisons by three multivariate statistical procedures showed that the specimens formed a complete cline of characteristics between *H. l. leonardus* and *H. l. pawnee* within several Iowa counties. All three analyses support north to south gradations from *H. l. pawnee* to *H. l. leonardus*, with populations in Monona, Harrison, and Pottawattamie counties in Iowa representing the greatest amount of intergradation. Results from this study support previous conclusions that *H. l. leonardus* and *H. l. pawnee* are conspecific.

Additional key words: intergrade, skipper, multivariate analysis, prairie, Iowa.

The Loess Hills are a series of loess covered bluffs formed by past action of winds on the Missouri River floodplain, extending approximately from the extreme southeastern corner of South Dakota to northwest Missouri (Fig. 1). The majority of these bluffs occur in Nebraska and Iowa, although by far the most extensive areas are in Iowa. The Loess Hills are composed of xeric, mixed-grass prairie, drought-tolerant forbs, and hardwood [e.g. bur oak (*Quercus macrocarpa* Michx., Fagaceae)] forests, invaded with red cedar (*Juniperus virginiana* L., Cupressaceae). Dominant grasses (Poaceae) include little bluestem (*Andropogon scoparius* Michx.), side-oats grama [*Bouteloua curtipendula* (Michx.) Torr.], and hairy grama (*B. hirsuta* Lag.). Although prairies dominate in the northern hill region, forests dominate in the southern hills, covering all but the driest, most exposed ridgetops and bluffs (Mutel 1989). A number of rare plants and animals reside in the Loess Hills.

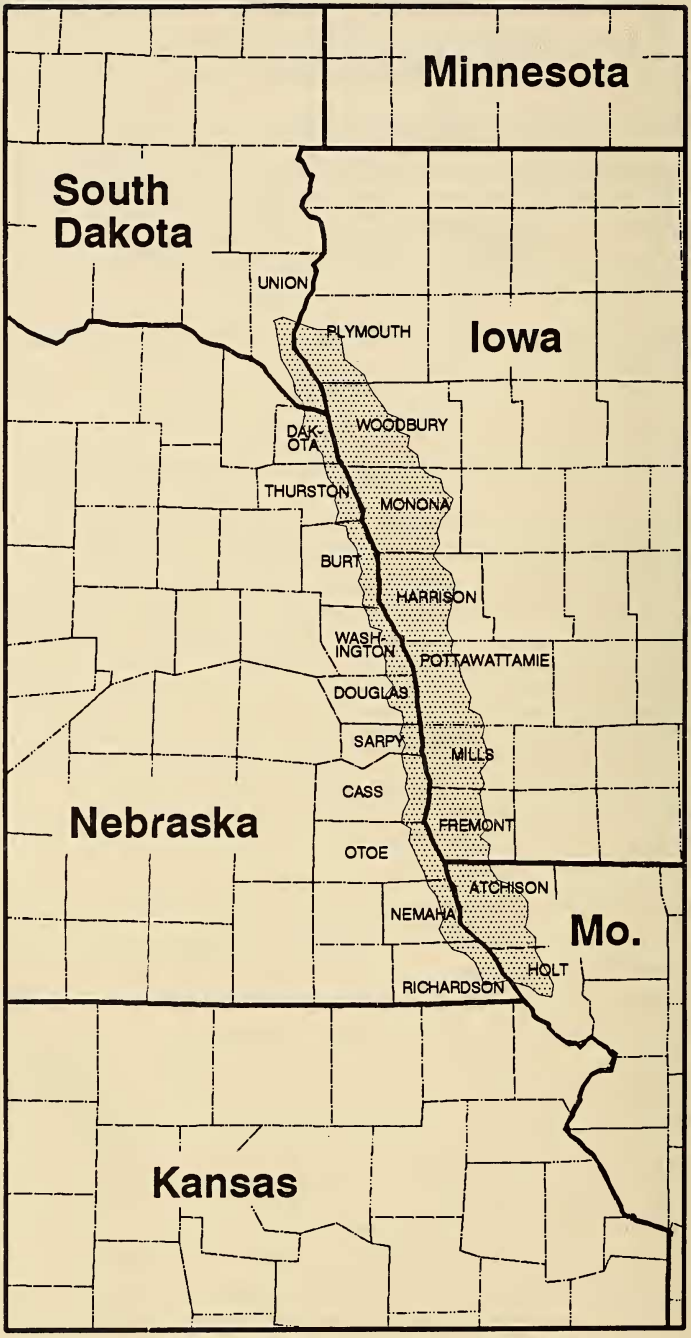

FIG. 1. Generalized geographic location of the Loess Hills.

Scott and Stanford (1981) concluded that Leonard's skipper (*Hesperia leonardus leonardus* Harris) and the pawnee skipper (*Hesperia leonardus pawnee* Dodge) are conspecific based primarily upon a series of intermediates from central Minnesota and a few specimens from adjacent states. Similarities in male genitalia, host plant requirements, and nectar sources between the two taxa also were mentioned. However, some authors still consider the two phenotypes to be distinct species, based on differences in habitat and appearance (Tilden & Smith 1986, Klassen et al. 1989).

Scott and Stanford (1981) suggested a blend zone between the two phenotypes. This blend zone included areas of southeastern Nebraska and western Iowa, part of which is encompassed by the Loess Hills. Unfortunately, very few specimens of *H. l. leonardus* or *H. l. pawnee* were known from the Loess Hills. Lindsey (1921) reported specimens from Sioux City, Iowa, probably collected in what is now Stone State Park in Plymouth and Woodbury counties, Iowa (W. W. McGuire, pers. comm.). Also, Barber (1894) cited a record of *H. leonardus* (sic *leonardius*) by W. E. Taylor from Nemaha Co., Nebraska that may have originated in the Loess Hills. In 1986, we rediscovered *H. l. pawnee* at Sioux City Prairie, and in 1988 we discovered a new colony in Union Co., South Dakota.

These recent discoveries of *H. l. pawnee* in the Loess Hills prompted us to survey additional sites within the Loess Hills. Our objectives were to (1) document the occurrence of *H. l. leonardus* and/or *H. l. pawnee* within the Loess Hills south of the previously mentioned sites, (2) search for identifiable intermediates that would substantiate the blend zone reported by Scott and Stanford (1981), and (3) record behavioral and ecological observations.

Materials and Methods

From 1989 through 1991, we conducted an intensive search of native prairie sites located within the Loess Hills from Union Co., South Dakota to Holt Co., Missouri. Adult *Hesperia* were collected and pertinent behavioral, biological, and ecological information was recorded.

Spread specimens were rated for five characteristics, as used by Scott and Stanford (1981), to distinguish between *H. l. leonardus* and *H. l. pawnee*. These five characteristics were: (1) forewing length, (2) ventral hindwing color [rated from 0 to 7 using eight standard reference specimens varying from golden (males) or greenish golden (females) in *H. l. pawnee* to dark reddish brown in *H. l. leonardus*], (3) ventral hindwing median band size (rated from 0 to 4 using five reference specimens varying from absence of band to large, distinct spots), (4) dorsal lightness of males (rated from 1 to 6 using six reference specimens varying from

light fulvous in *H. l. pawnee* to very dark in *H. l. leonardus*), and (5) transparency of the dorsal forewing hyaline spot in females (rated from 1 to 4 using four reference specimens varying from white in *H. l. pawnee* to dark yellow in *H. l. leonardus*). Reference specimens also included examples of typical *H. l. pawnee* from west-central Nebraska and Pipestone Co., Minnesota, and typical *H. l. leonardus* from the Missouri Ozarks. Reference specimens also were rated for the five variables listed above. Two additional variables used by Scott and Stanford (1981), dorsal lightness of females and darkness of ventral forewing tornus, were not used because they were too variable to be useful for comparative purposes, even among typical *H. l. pawnee* individuals.

We used three multivariate statistical procedures to identify relationships between specimens at different locations (counties). Specific analyses included cluster analysis, canonical discriminant analysis, and discriminant analysis based on the observed variable (SAS Institute 1988). Because classification criteria differed by sex, all analyses were conducted separately for males and females. Cluster analysis, using the average linkage method, was conducted on specimens from both the potential intergrade zone (Loess Hills) and the reference specimens. Canonical discriminant analysis also was used for these (Loess Hills and reference) specimens to provide an alternative procedure for classification. For the final procedure, discriminant analysis, specimens from Union, Plymouth, and Woodbury counties in Iowa were classified as *H. l. pawnee* and those from Mills and Fremont counties in Iowa as *H. l. leonardus*. These were then used as a known data set to discriminate specimens as *H. l. pawnee* or *H. l. leonardus* for all eight Loess Hills counties.

Results and Discussion

By 1991, we had investigated nearly every Loess Hills county in Nebraska and all Loess Hills counties in South Dakota, Iowa, and Missouri. New populations of *H. l. leonardus/pawnee* were discovered and collected in Monona, Harrison, Pottawattamie, Mills, and Fremont counties in Iowa. No specimens were found in Nebraska or Missouri. A total of 208 Loess Hills specimens, representing 43 sites, was rated. Densities of *H. leonardus* varied between sites, appearing to be influ-

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FIG. 2. Loess Hills specimens of *Hesperia leonardus*. Column 1 = Union Co., South Dakota; column 2 = Plymouth and Woodbury co.'s, Iowa; column 3 = Monona Co., Iowa; column 4 = Harrison Co., Iowa; column 5 = Pottawattamie Co., Iowa; column 6 = Mills Co., Iowa; column 7 = Fremont Co., Iowa.

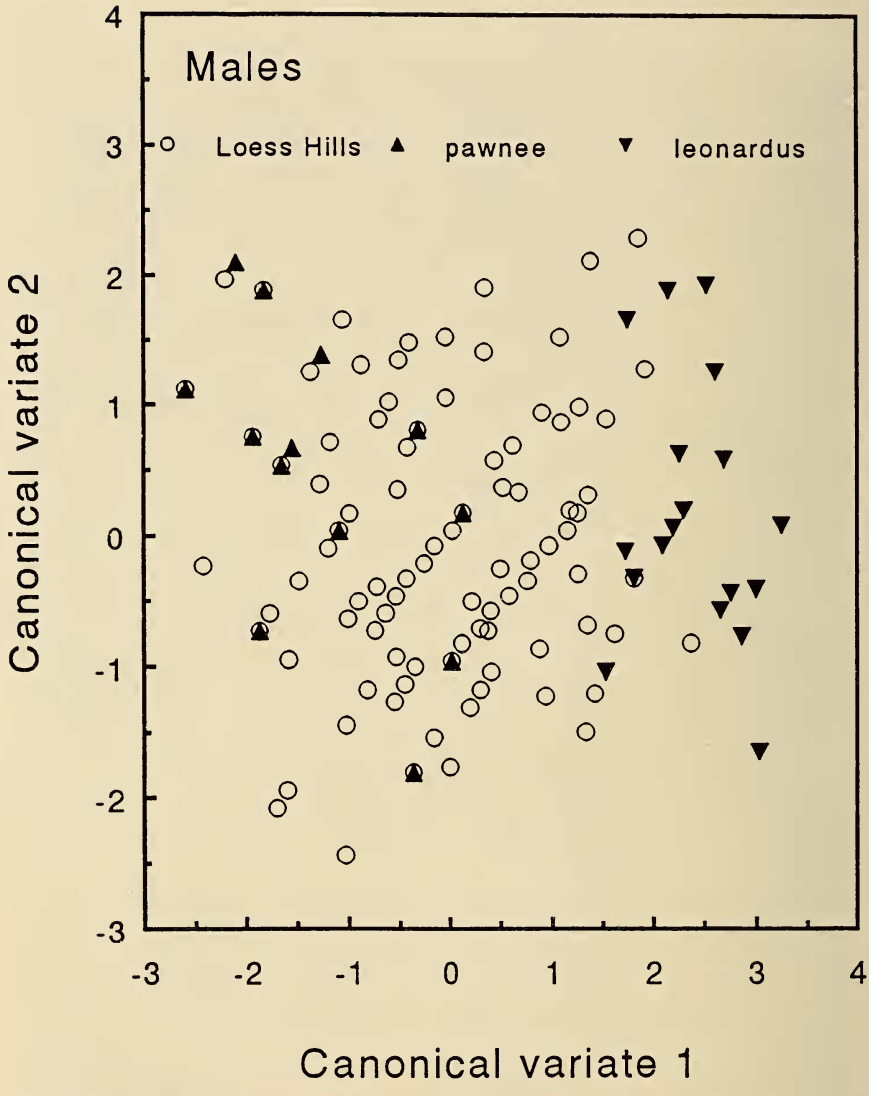


FIG. 3. Results of the canonical discriminant analysis of *Hesperia leonardus* populations—males.

enced most by habitat quality and availability of blazing star (*Liatris punctata* Hook., Asteraceae), an important nectar source.

Loess Hills specimens of *H. leonardus* showed a complete cline of characteristics from typical *H. l. pawnee* in the northern Hills, to typical (Ozark) *H. l. leonardus* in the south (Fig. 2). Populations from Monona,

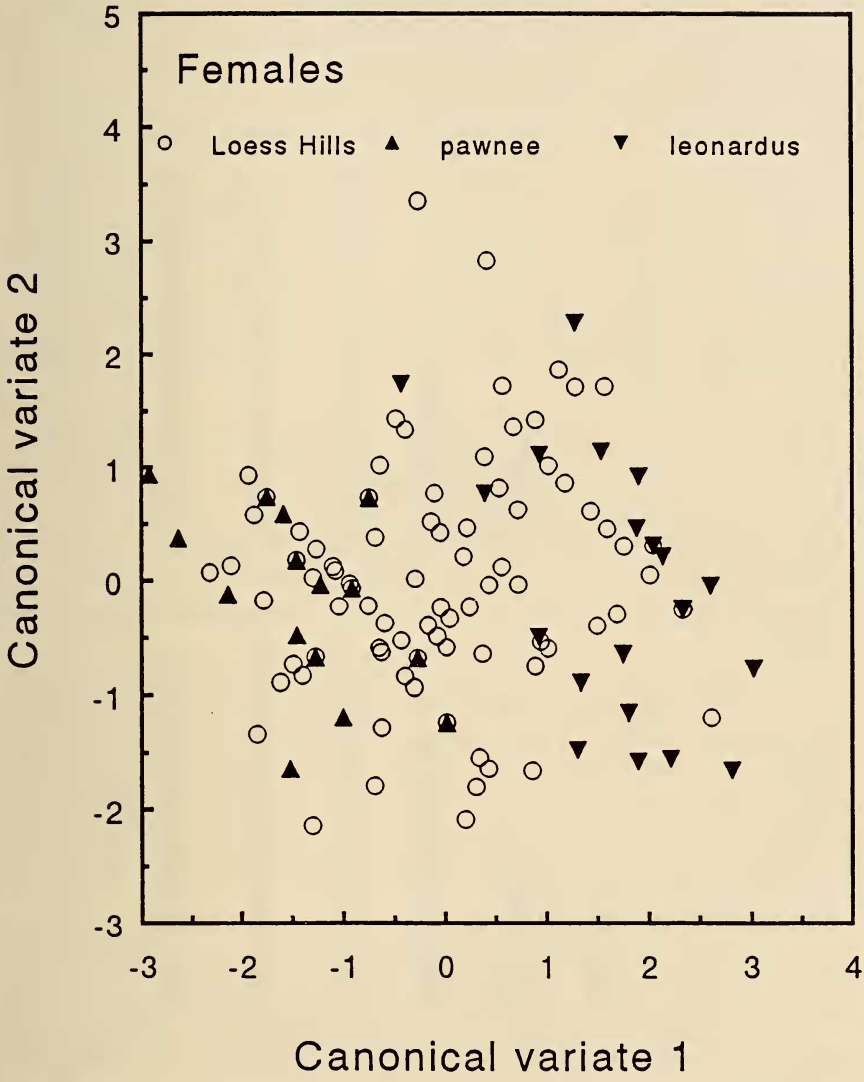


FIG. 4. Results of the canonical discriminant analysis of *Hesperia leonardus* populations—females.

Harrison, and Pottawattamie counties in Iowa showed the greatest degree of intergradation between the two taxa.

The results of the canonical discriminant analyses are shown in Figs. 3 and 4. The first canonical variate provided the most significant discrimination of specimens for both sexes ($P > 0.0001$). The second

canonical variate was not significant for either sex ($P > 0.3949$ males, $P > 0.9095$ females) but is used in Figs. 3 and 4 for ease of viewing individual points. (In other words, only the X-axis spacing of points of Figs. 3 and 4 represents significant discrimination of specimens.) Ventral hindwing median band size, fulvousness of males, ventral hindwing color, and transparency of the dorsal forewing hyaline spot in females were the most important canonical coefficients. Although both sexes of Loess Hills specimens overlapped the variation found in the reference specimens of typical *H. l. leonardus* and *H. l. pawnee*, females showed the greatest amount of variation (Fig. 3). For both sexes, Loess Hills specimens were ranked between known *H. l. leonardus* and *H. l. pawnee* specimens, although males overlapped more with *H. l. pawnee* than *H. l. leonardus*.

Results of the discriminant analyses are shown in Fig. 5. These analyses supported ranking material from Union, Plymouth, and Woodbury counties as *H. l. pawnee* and from Mills and Fremont counties as *H. l. leonardus*. Additionally, a north to south gradation from *H. l. pawnee* to *H. l. leonardus* is evident, with the greatest incidence of intermediate specimens (those with a <95% probability of being *H. l. pawnee* or *H. l. leonardus* by this analysis) in Monona, Harrison, and Pottawattamie counties.

Dendograms constructed from the cluster analyses are shown in Figs. 6 and 7. Although clusters essentially agree with the other analyses, intergrade populations from Harrison, Monona, and Pottawattamie counties are closer to *H. l. pawnee* than to *H. l. leonardus* (especially males) by this analysis. A sample of four males from Lincoln Co., Nebraska (west-central Nebraska) also clustered with the Loess Hills intergrades, apparently because of their tendency towards a darker, or less fulvous, dorsal phenotype.

All three analyses support north-to-south gradations from *H. l. pawnee* to *H. l. leonardus*, with populations in Monona, Harrison, and Pottawattamie counties representing the greatest amount of intergradation. Although some differences were noted between sexes, trends toward intergradation by location generally agreed for both males and females.

These analyses support the assertion by Scott and Stanford (1981) that *H. l. leonardus* and *H. l. pawnee* are subspecies rather than species. Other observations during this study support this conclusion. For example, a sample of male genitalia, representing typical *H. l. pawnee*, *H. l. leonardus*, and Loess Hills specimens was examined. MacNeill (1964) described differences in genitalia between *H. l. leonardus* and *H. l. pawnee*; however, Scott and Stanford (1981) stated that the genitalia of the two were too variable to detect differences. We found that

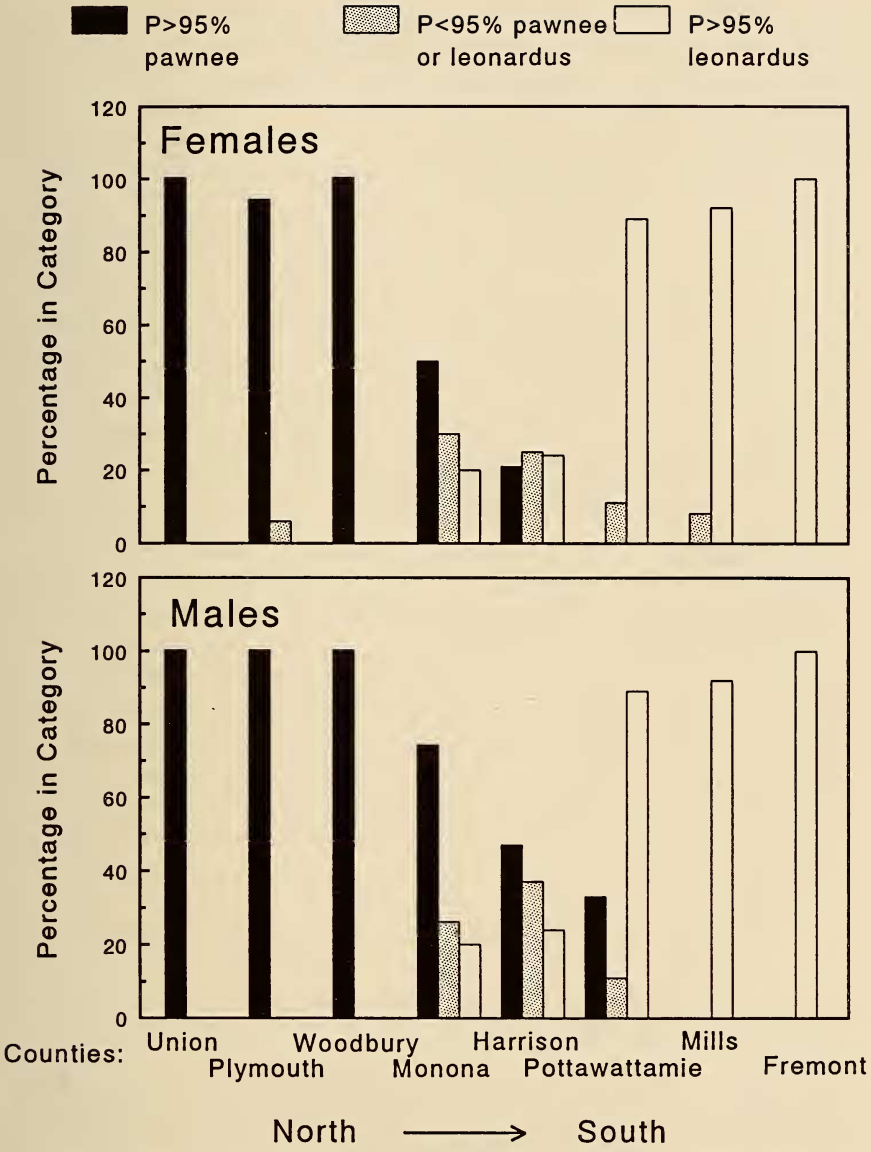
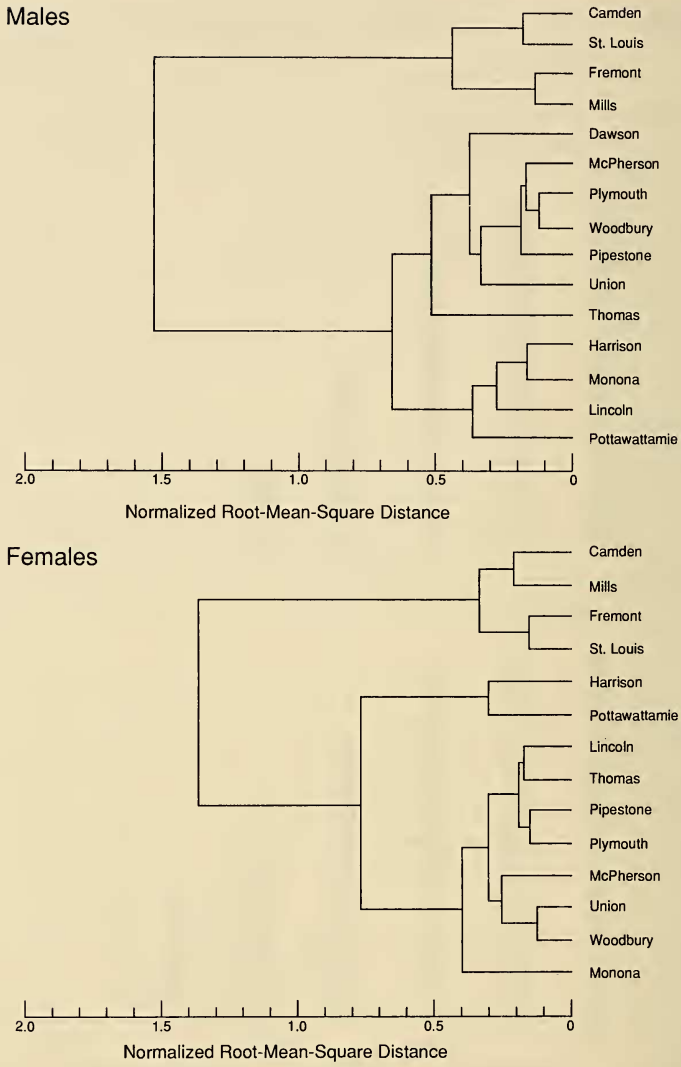


FIG. 5. Results of the discriminant analysis for male and female *Hesperia leonardus*, indicating probability of membership in a given subspecies by county (see text for details).

the degree of variability (even within one population) was too high to consider using male genitalia as a diagnostic character.

The major nectar source of the Loess Hills *H. leonardus* is blazing star, although rough gayfeather (*Liatris aspera* Michx., Asteraceae),



FIGS. 6 and 7. Dendograms of the cluster analysis of *Hesperia leonardus* populations. **6.** Males; **7.** Females. Code for counties listed above: Camden, St. Louis = Missouri Ozarks (*leonardus*); Dawson, McPherson, Thomas, Lincoln = Nebraska (*pawnee*); Pipestone = Minnesota (*pawnee*); Union = South Dakota; Plymouth, Woodbury, Monona, Harrison, Pottawattamie, Mills, Fremont = Iowa (Loess Hills populations).

annual sunflower (*Helianthus annuus* L., Asteraceae), and thistle (*Cirsium* spp., Asteraceae) were used in the absence of *L. punctata*. All specimens from the Loess Hills showed a preference for *L. punctata* when more than one nectar source was available.

The typical habitat for Loess Hills *H. leonardus* populations was ridgetop prairie; however, specimens also were found in roadside ditches at the base of the hills in Mills and Fremont counties. Although it has been reported that *H. l. leonardus* males choose higher perches than western populations [i.e., *H. l. pawnee* and *H. l. montana* (Skinner)] (Scott and Stanford 1981), this is more than likely a function of availability of high vs. low perching sites in a particular location. We observed no differences in perching sites between *H. l. pawnee* in the northern Hills and *H. l. leonardus* in the southern Hills.

The larval host of the Loess Hills *H. leonardus* populations remains unknown. In 1989, one adult female was observed crawling around the base of little bluestem, presumably to oviposit. Recently, Wooley et al. (1991) reported that blue grama [*B. gracilis* (H.B.K.) Griffiths] was the only observed larval host for the subspecies *H. l. montana* in Colorado. Although there is little blue grama in the Loess Hills, there are sufficient stands of side-oats grama and hairy grama to provide larval resources for *H. leonardus*.

The exact factors influencing the distribution and phenotypic expression of *H. l. pawnee* and *H. l. leonardus* in the Loess Hills are unknown. However, the north-to-south transition from *H. l. pawnee* to *H. l. leonardus* appears to be a case of primary intergradation: relatively smooth character clines between contiguous populations in continuous contact (Mayr 1963). Primary intergradation is believed to be caused by corresponding changes in environmental conditions, which fits conditions associated with the variation between *H. leonardus* subspecies in this study. The scarcity of ridgetop prairies in the southern Loess Hills may be preventing the southward spread of *H. leonardus* because of fragmentation of habitat and hosts. Prairies probably were once continuous or nearly so in the Loess Hills from north to south before the elimination of prairie fires and the subsequent accelerated invasion of trees and woody shrubs. The southern Loess Hills populations of *H. l. leonardus* may have been connected to Ozark populations before disruption of habitat occurred. Increased humidity and rainfall also are associated with the increased forestation in the southern Loess Hills. The mean annual temperature also increases from north to south along the Loess Hills. Possibly humidity, or a combination of temperature and humidity, has contributed to the divergence of *H. l. pawnee* to the north and *H. l. leonardus* to the south.

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